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(54) Title: METHOD FOR DELIVERY OF THERAPEUTIC AGENTS TO TARGET BRAIN TISSUE USING MONOCLONAL ANTIBODY CONJUGATES

(57) Abstract

A method for the delivery of therapeutic agents into the brain is disclosed. A selected chemical agent is first administered into the blood stream of a subject in order to increase blood brain barrier (BBB) permeability. Thereafter, a chemical conjugate consisting of a selected monoclonal antibody in combination with an enzyme is administered. The conjugate passes through the BBB and selectively binds to brain lesion/tumor tissue. The BBB is then allowed to return to ambient permeability levels, wherein residual circulating amounts of the conjugate in the subject are removed through renal or other clearance mechanisms. Next, a selected prodrug is administered having a molecular weight sufficiently small to pass through the BBB. The prodrug is reacted upon by the enzyme to form a therapeutic drug, such formation being directly at the site of the brain lesion/tumor proliferation.

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METHOD FOR DELIVERY OF THERAPEUTIC AGENTS TO TARGET BRAIN TISSUE USING MONOCLONAL ANTIBODY CONJUGATES

This invention was made with Government support from the Veterans Administration and under Grant No. 31770 from the National Institutes of Health. The Government has certain rights in this invention.

Background of the Invention

The present invention generally relates to the treatment of brain tissue disorders, and more particularly to a method for treating such disorders using monoclonal antibody technology.

In treating diseases of the brain and central nervous system, especially those involving neoplastic tissue growth, a practical method for the delivery of drugs across the blood brain barrier (BBB) is needed. The BBB is a capillary barrier comprising a continuous layer of tightly bound endothelial cells. These cells permit a low degree of transendothelial transport, and exclude molecules in the blood from entering the brain on the basis of molecular weight and lipid solubility, as described in Neuwelt, E.A., "Is There A Therapeutic Role For Blood-Brain Barrier Disruption", Ann. Int. Med. 93:137-139, 1980. For example, the BBB normally excludes molecules with a molecular weight greater than 180 daltons. In addition, the lipid solubility of molecules is a major controlling factor in BBB passage.

Considerable research has been conducted relating to the BBB and its permeability. Articles generally involving permeability of the BBB include:

1. Greig, N.H., "Chemotherapy of brain metastases: Current status", Cancer Treatment Reviews, 11:157-186 (1984).

2. Neuwelt, E.A., et al, "Cerebrovascular permeability and delivery of gentamicin to normal brain and experimental brain abscess in rats", Journal of Neurosurgery, 61:430-439 (1984).

3. Sage, M.R., "Blood-Brain Barrier: Phenomenon of Increasing Importance to the Imaging Clinician", American Journal of Roentgenology, 138:887-898 (1982).

4. Hiesinger, E.M. et al, "Opening the Blood-Brain and Blood-Tumor Barriers in Experimental Rat Brain Tumors: The Effect of Intracarotid Hyperosmolar Mannitol on Capillary Permeability and Blood Flow", Annals of Neurology, 19:50-59 (1986).

The articles listed above discuss BBB permeability characterisics in terms of lipid solubility, ionization fraction, protein binding and/or the molecular weight of foreign molecules.

As specifically described by Sage, supra, the function of the BBB is to maintain the homeostasis of the neuronal environment. The continuity produced by the tight junctions between individual cells of the BBB enables the cerebrocapillary endothelium to act like a plasma membrane. Small molecules (m.w. <200 daltons) having a high degree of lipid solubility and low ionization at physiological pH are freely passed through the BBB. In addition, the BBB allows water to move in either direction in order to maintain equal osmotic concentrations of solutes in the extracellular cerebral fluid.

Thus, the permeability characteristics of the BBB must be considered in treating central nervous system disorders. While the interendothelial junctions between the cells of the BBB are normally

designed to keep potentially noxious substances away from the brain, chemicals exist which may osmotically disrupt the BBB, thereby increasing its permeability. Exemplary materials for this purpose include hypertonic solutions of mannitol, arabinose and/or glycerol. Chemical disruption of the BBB has been characterized in a variety of articles, including Neuwelt, E. A., et al, "Osmotic Blood-Brain Barrier Opening to IgM Monoclonal Antibody in the Rat", Am. J. Physiol., 250:R875-883, 1986.

By chemically disrupting the BBB, large molecules have been transferred across the BBB in animal subjects. For example, in Neuwelt, E.A., et al, "Osmotic blood-brain barrier opening to IgM Monoclonal Antibody in the Rat", supra, monoclonal antibodies Ab348 624E5 and Ab 350 624H12 [both rat immunoglobin M(IgM)] were administered to adult Sprague-Dawley rats after the administration of mannitol solutions. Osmotic BBB opening in this manner significantly increased monoclonal antibody uptake into the rat brain tissues.

In addition, tests have also indicated that BBB permeability may change upon the formation of brain abscesses, inflammation, and/or tumors. Under these conditions, BBB permeability has been shown to increase. This increase frequently allows the greater influx of molecules having a relatively small molecular weight (<1,000 daltons) which would nonetheless be excluded under normal BBB conditions. For example, experimental allergic encephalomyelitis (EAE) may cause an immune reaction which increases BBB permeability. Alvoode, E.C. et al, "Experimental Allergic Encephalomyelitis: A Useful Model For Multiple Sclerosis", Prog. Clin. Biol. Res., Vol. 146, Alan R. Liss Co., N.Y., 1984. One explanation for the increased permeability of the BBB at the onset of EAE

involves the capability of endothelial cells of the cerebrovasculature system to act as antigen presenting cells (APCs), thus attracting T-cells and aiding their penetration across the BBB.

Another possible explanation for the increase in BBB permeability during the onset of cerebral lesions involves the ability of the brain under adverse circumstances to generate vasoactive substances, as described in Black, K.L., "Leukotrienes Increase Blood-Brain Barrier Permeability Following Intraparenchymal Injections In Rats", Ann Neurol., 18:349-351, 1985. Brain lipids are rich in arachidonic acid which may be released by brain tissue in response to trauma, neoplastic invasion or ischemia. Black demonstrated experimentally that arachidonic acid and leukotrienes can increase BBB permeability when injected directly into the rat brain. Leukotriene content of the brain tissue correlates significantly with the amount of edema surrounding various CNS neoplasms, and it is conceivable that leukotrienes released from the damaged brain contribute to BBB disruption and vasogenic edema in CNS neoplasia.

Likewise, inflammation of brain tissue in immune-mediated CNS diseases might possibly cause release of arachidonic acid and leukotrienes which would increase BBB permeability. A further discussion of increased BBB permeability with reference to nervous system disorders including infections, inflammatory conditions, neoplasms, and ischemia is presented in Fishman, R.A., Cerebrospinal Fluid in Diseases of the Nervous System, W. B. Saunders Co., Philadelphia, London, Toronto, 1980; Tourtelotte, W. "On Cerebrospinal IgG Quotients In Multiple Sclerosis and Other Diseases. A Review And A New Formula To Estimate The Amount Of IgG Synthesized Per Day By the

Central Nervous System", J. Neurol. Sci., 10:279-304, 1970.

Notwithstanding the advances described above, a need exists for an effective way to deliver therapeutic agents across the BBB in order to treat brain disorders. The present invention satisfies this need using chemical BBB modification in combination with monoclonal antibody technology in a manner not heretofore known in the art.

Summary of the Invention

It is an object of the present invention to provide a method for the delivery of thereapeutic agents into the brain in order to treat brain lesions (i.e., tumors, abscesses and similar disorders).

It is another object of the invention to provide a method for the delivery of therapeutic agents into the brain in which BBB permeability is modified by the administration of chemicals designed to increase BBB permeability.

It is a further object of the invention to provide a method for the delivery of therapeutic agents into the brain which utilizes monoclonal antibodies to target specific brain lesions or regions (i.e. the basal ganglia in movement disorders such as Parkinsonism).

It is an even further object of the invention to provide a method for the delivery of therapeutic agents into the brain which enables the delivery of a prodrug across the BBB, followed by site-specific enzymatic conversion of the prodrug to an active drug having substantial therapeutic activity.

To accomplish these objectives, a method for the delivery of therapeutic agents across the BBB is disclosed which uses monoclonal antibody technology in

combination with chemical modification of the BBB. In order to deliver therapeutic agents into the brain, a chemical composition is first administered which increases BBB permeability. Thereafter, a chemical conjugate consisting of a monoclonal antibody in combination with an enzyme is administered. The conjugate passes through the BBB and binds to neoplastic tissue in the brain or other disease-affected areas. Thereafter, the BBB permeability is allowed to return to pre-treatment levels, wherein residual circulating amounts of the conjugate in the subject outside of the BBB are removed through renal clearance or other mechanisms. Next, a selected prodrug is administered to the subject having a molecular weight sufficiently small to pass through the BBB which, in many CNS lesions, is differentially permeable to smaller molecules as opposed to the high molecular weight conjugates described herein. The conjugate enzyme acts on the prodrug to form a therapeutically effective drug useful in treating the brain lesions of concern.

These and other objects, features, and advantages of the invention will be described below in the following detailed description of a preferred embodiment.

Detailed Description

The present invention involves a method for treating brain lesions. As used herein, the term "lesions" shall encompass malignant tumors, CNS infections, brain abscesses, cerebro-vascular disorders, and even degernative disorders such as Parkinson's disease and Alzheimer's disease. These disorders have many causes, including bacterial/viral infection or problems of genetic origin. Blood brain

barrier (BBB) permeability in a warm blooded subject is first modified through the administration of a suitable chemical agent. For the purposes of this application, the term "BBB" shall also encompass the vascular barrier associated with any brain tumors/lesions, otherwise known as the "blood-brain lesion barrier." Chemical agents useful for this purpose include hypertonic solutions of mannitol, arabinose, glycerol, and others known in the art. Administration is generally accomplished by intra-arterial infusion techniques known in the art. As a result, BBB permeability will be greatly increased. For example, a normal, unmodified BBB will prevent molecules larger than 180 daltons from entering the brain. After the administration of suitable agents, the BBB will permit the passage of molecules having a molecular weight of 1,000,000 daltons. Preliminary studies indicate that even viral particles can be delivered across the BBB in this way.

Increased BBB permeability enables administration of therapeutic agents or drugs which would not normally pass through the BBB. In the present invention after BBB modification, a chemical conjugate is administered consisting of a monoclonal antibody in combination with a selected enzyme. Considerable research has been conducted involving tumor-associated monoclonal antibody conjugates, as discussed in Moller, G., "Antibody Carriers of Drugs and Toxins in Tumor Therapy", Immuno. Rev., 62, 1982 and Senter, P.D., et al, "Antitumor Effects of Antibody-Alkaline Phosphatase Conjugates in Combination With Etoposide Phosphate", Proc. Natl. Acad. Sci. (In Press). Recent developments in this area have been made possible by monoclonal antibodies which recognize cell-surface antigens relative to a variety of carcinomas, melanomas, and lymphomas.

According to Senter, et al, monoclonal antibodies have been used in the past as carriers for many important anticancer agents.

In the present invention, a selected monoclonal antibody is combined with an enzyme to form a conjugate of relatively high molecular weight which is administered to the subject following BBB modification. Normally, the high molecular weight conjugate would not readily pass through an unmodified BBB. However, entry of the conjugate into the brain is possible when BBB permeability is modified as described above.

Upon administration of the conjugate and passage thereof through the modified BBB, the conjugate binds to the surface of antigen positive lesion/tumor cells within the brain. Thereafter, the BBB is allowed to return to pre-modification levels as the effects of the previously-delivered chemical agent completely reverse. Typically this will occur spontaneously in about thirty minutes.

Next, a selected prodrug is administered intra-arterially or by other parenteral routes. For the purposes of this invention, a "prodrug" is defined as an active drug linked to a moiety which is then metabolized to the active agent <u>in vitro</u> and/or <u>in vivo.</u> The prodrug will preferably have a molecular weight small enough (i.e. 250 - 1,000 daltons) to pass through an unmodified differentially permeable blood-brain or blood-brain lesion barrier. As previously discussed, ambient (i.e., pre-treatment) BBB permeability is somewhat higher during the manifestation of brain lesions and similar conditions. This enables certain prodrugs to more readily pass through the BBB.

After passing into the brain, the prodrug is chemically altered by the enzyme in the conjugate. As a result, the prodrug is enzymatically modified to produce a therapeutically effective drug which is capable of diminishing or controlling tumor development or other brain tissue disorders.

A specific embodiment of the foregoing process is presented in the following Example:

EXAMPLE

In the article by Senter, et al, (attached as Exhibit A and incorporated herein by reference) information is provided regarding a monoclonal antibody/enzyme conjugate used to control tumor growth. Specifically, a conjugate was formed involving the L6 (IgG_{2a}) monoclonal antibody. Previous tests on L6 indicated that it was capable of binding to a carbohydrate antigen on human lung carcinoma, as discussed in Hellstrom, I., et al, Cancer Res. 46:3917-3923 (1986).

To form the conjugate, L6 was combined with the enzyme alkaline phosphatase (A.P.). According to Senter, et al supra, the resulting conjugate is capable of converting the prodrug etoposide phosphate (E.P.) into etoposide. Etoposide (4'-Demethyl-epipodophyllotoxin 9-[4,6-0-(R)-ethylidene-Beta-D-glucopyranoside]) is a known drug with significant antineoplastic capabilities.

As further described in Senter et al, supra, the L6-A.P. conjugate was specifically prepared by modifying the L6 monoclonal antibody with iminothiolane (0.5mM) in order to add a single thiol group onto the L6 molecule. The A.P. used to form the conjugate was obtained from calf intestine having a molecular weight of 140kDa. Prior to conjugate

formation, the A.P. was modified with succinimidyl-4(N-maleimidomethyl) cyclo-hexane-1-carboxylate. The A.P. and L6 were then combined and the resulting conjugate products purified by gel filtration on S-300 Sephacryl.

The E.P. prodrug was prepared by the phosphorylation of etoposide (obtained from the Bristol-Meyers Co.) using an equimolar amount of phosphoryl chloride and acetonitrile and N,N-diisopropyl ethyl amine. The intermediate product was then hydrolyzed with aqueous $NaHCO_3$ and purified on a C-18 silica gel column. The column was washed with water, and the product eluted with 20% methanol in water.

The completed conjugate was tested using mice. Also, in vitro experiments were conducted using a cell line designated "H3347" which was obtained from the Oncogen Company. This cell line was established from a metastatic human colon carcinoma.

With respect to the in vitro tests, a suspension of 10^6 H3347 cells in 0.1 ml of incomplete modified Delbecco's medium (IMDM) with 10% fetal calf serum was maintained at 4°C for a one-hour period in combination with 5ug/ml of the L6-A.P. conjugate. The cells were washed twice with the IMDM, resuspended, and plated into 96-well microtiter plates (10,000 cells/well). The E.P. in IMDM was then added to the cells, and the mixture incubated at 37°C for six hours. Thereafter, the cells were washed twice and incubated for twelve more hours. The cells were then subjected to a six hour pulse with ^{3}H-Thymidine (1.0uCi/well). Next, the plates were frozen at -20°C, and the cells harvested onto glass fiber discs.

The in vitro cytotoxic effects of the enzymatically produced etoposide were determined by measuring the incorporation of ^{3}H-Thymidine into the

DNA of the H3347 cells. Decreased ^{3}H-Thymidine uptake (i.e., decreased DNA replication) indicated an increase in cytotoxicity which in turn demonstrated a decrease in cell growth. Accordingly, the test results showed a substantial increase in cytotoxic activity when the cells were exposed to the conjugate, and then to the E. P.

With respect to the in vivo studies, Balb C nu/nu female mice (four to six weeks old) were injected with 10^{7} H3347 cells subcutaneously in the left and right flanks. The tumor cells used in these experiments were obtained from in vitro cultures previously suspended by treatment for two minutes with trypsin (0.5 g/l) and EDTA (0.2 g/l).

The L6-A.P. conjugates (0.1 ml containing 300ug monoclonal antibody in PBS) and etoposide phosphate (0.2 ml containing 2 mg E.P. in water) were then administered to the mice interperitoneally. The L6 A.P. was given 18-24 hours prior to treatment with the E.P. Tumor growth in the mice was compared to growth in untreated mice and mice treated with maximum tolerated doses of etoposide or etoposide phosphate alone. The results indicated that etoposide alone had very little effect on tumor growth, and higher doses were not well tolerated. The E.P. was less toxic to the animals and exhibited a greater antitumor effect than treatment with etoposide alone. However, the greatest anti-tumor effects were observed in mice treated with the conjugate followed by E.P.

The experiments by Senter et al clearly demonstrate the effectiveness of a monoclonal antibody/alkaline phosphatase conjugate used to produce etoposide from etoposide phosphate. It is predicted that these principles and materials may be applied to brain tissues for the treatment of lesions

(tumors, etc.) in accordance with the present invention.

The BBB would first be opened by administration of a hypertonic solution capable of increasing BBB permeability. Exemplary solutions for this purpose would include hypertonic mannitol, glycerol, and/or arabinose as previously discussed. Thereafter, a conjugate consisting of IgG_{2a} monoclonal antibody in combination with alkaline phosphatase would be injected into the subject. The conjugate would pass through the modified BBB and bind to the lesion/tumor tissues. Sufficient time would then be allowed for the BBB to return to pre-treatment permeability levels. Any remaining conjugate outside the BBB would be cleared from the subject either renally, or through other mechanisms. For example, clearance could occur via the reticulo-endothelial system, or through the addition of antibodies designed to enhance clearance, as is generally discussed in Sharkey, R.M. et al, "Factors Influencing Anti-Antibody Enhancement of Tumor Targeting with Antibodies in Hamsters with Human Colonic Tumor Xenografts", Cancer Research, 48:2005-2009 (1988). Next, E.P. would be administered which would pass through the BBB because of its relatively low molecular weight. Once within the BBB, the etoposide phosphate would be converted to etoposide primarily in the regions of lesion/tumor proliferation.

The foregoing process offers numerous benefits. It is capable of selectively delivering high-molecular-weight materials across the BBB which may be used to convert a prodrug into an active therapeutic agent. Furthermore, the therapeutic agent would be delivered directly to the site of the lesion/tumor proliferation. It would not be necessary for the monoclonal antibody conjugate to bind to every

lesion or tumor cell, since the resulting drug formed through enzyme prodrug conversion could diffuse directly to adjacent cells.

Having herein described a preferred embodiment of the invention, it is contemplated that suitable modifications may be made thereto by those skilled in the art. Thus, it is anticipated that the invention shall only be construed in accordance with the following claims:

WHAT IS CLAIMED IS:

1. A method for the delivery of therapeutic agents into the brain of a warm blooded animal for the treatment of brain lesions comprising the steps of:

administering a chemical agent to said animal capable of increasing the permeability of the blood brain barrier of said brain from an initial permeability level to a level of increased permeability;

administering a chemical conjugate to said animal comprising at least one monoclonal antibody in combination with at least one enzyme, said conjugate passing through said blood brain barrier because of the increased permeability thereof, said conjugate thereafter binding to the tissues of said brain lesions;

allowing said blood brain barrier to return to said initial permeability level so that said brain may function normally; and

administering at least one prodrug to said animal having a molecular weight sufficiently small to pass through said blood-brain barrier, said prodrug being reacted upon by said enzyme in said conjugate in order to form an active drug therapeutically effective in the treatment of said brain lesions.

2. The method of claim 1 wherein said chemical agent comprises a hypertonic solution of a material selected from the group consisting of mannitol, arabinose, and glycerol.

3. The method of claim 1 wherein said enzyme of said chemical conjugate comprises alkaline phosphatase.

4. The method of claim 1 wherein said monoclonal antibody of said conjugate comprises IgG_{2a}.

5. The method of claim 1 wherein said prodrug has a molecular weight of about 200 to 1,000 daltons.

6. The method of claim 1 wherein said prodrug comprises etoposide phosphate, said etoposide phosphate being reacted upon by said enzyme in said conjugate to form etoposide.

7. The method of claim 1 wherein said blood-brain barrier is a blood-brain lesion barrier.

8. A method for the delivery of therapeutic agents into the brain of a warm blooded animal for the treatment of brain lesions comprising the steps of:

administering a chemical agent to said animal capable of increasing the permeability of said blood brain barrier of said brain from an initial permeability level to a level of increased permeability, said chemical agent comprising a hypertonic solution of a material selected from the group consisting of mannitol, arabinose, and glycerol;

administering a chemical conjugate comprising the monoclonal antibody IgG_{2a} in combination with alkaline phosphatase, said conjugate passing through said blood brain barrier because of the increased permeability thereof, said conjugate thereafter binding to the tissues of said brain lesions;

allowing said blood brain barrier to return to said initial permeability level so that said brain may function normally; and

administering at least one prodrug comprising etoposide phosphate to said animal, said etoposide phosphate being reacted upon by said alkaline

phosphatase in said conjugate in order to form etoposide, said etoposide being therapeuticlly effective in the treatment of said brain lesions.

5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/02110

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL(4): A61K 49/00; G01N 33/48

U.S. CL: 424/9, 85.91, 94.3

II FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/9, 85.91, 94.3; 435/7; 436/519

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Chemical Abstracts (on line) 1967-present; BIOSIS (on line) 1975-present; Medline 1975-present; U.S. Automated Patent System 1975-present

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,479,932 (Bodor) 30 October 1984, see column 4, line 67-column 5, line 26.	1,3,5-6,8
Y	Chemical Abstracts, Vol. 107, No.19, issued 9 November 1987, pages 328, abstract No. 171555s, Blasberg et al, "Regional Localization of a Glioma-Associated antigen defined by Monoclonal Antibody 81C6...", see whole abstract.	1,4-5,7-8
Y	Proceedings of the National Academy of Science USA, Vol. 76, No. 1, issued January 1979, Barranger et al, "Modification of the Blood-Brain Barrier...", pages 481-485, see abstract, page 481, column 1, paragraph 2; page 484 column 2, three full paragraphs.	1-5,8
Y	American Journal of Physiology, Vol. 250, issued 1986, Neuwelt et al, " Osmotic Blood-Brain Barrier opening to IgM Monoclonal Antibody in the Rat", pages R875-R883, see abstract.	1-2,4-5, 7-8

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15 August 1989	26 SEP 1989
International Searching Authority	Signature of Authorized Officer
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